Dengue Virus 1 Outbreak in Buenos Aires, Argentina, 2016

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The largest outbreak of dengue in Buenos Aires, Argentina, occurred during 2016. Phylogenetic, phylodynamic, and phylogeographic analyses of 82 samples from dengue patients revealed co-circulation of 2 genotype V dengue virus lineages, suggesting that this virus has become endemic to the Buenos Aires metropolitan area.

Dengue virus (DENV) is a single-stranded RNA flavivirus primarily transmitted among humans by the *Aedes aegypti* mosquito. A substantial increase in dengue incidence has been observed in the past 2 decades in the Americas, and most of Argentina's bordering countries have reported co-circulation of >1 dengue serotype (*1*). In Buenos Aires, Argentina, the presence of the *A. aegypti* mosquito vector has been reported since 1995; DENV-1 local transmission was detected for the first time in 2009. No new autochthonous cases were detected again until 2016, when the worst dengue outbreak in decades occurred in the Americas (*2,3*).

The Study

During December 2015–April 2016, we confirmed 2,306 cases of human infection with DENV-1 in the virology laboratory at Hospital de Niños R. Gutiérrez. Most (84.69%) cases occurred during February and March. Patient ages ranged from 0 to 93 years (median 30 years). Of the 2,306 laboratory-confirmed cases, 76.7% of patients reported no recent travel history outside the Buenos Aires metropolitan area within 15 days before the onset of fever (local cases). The remaining cases had recently returned from dengue-affected areas (imported cases).

To characterize the outbreak, we sequenced the DENV-1 envelope glycoprotein (E) gene of 82 positive samples

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from local and imported cases (online Technical Appendix Table, https://wwwnc.cdc.gov/EID/article/23/10/16-1718-Techapp1.pdf). We used 3 phylogenetic inference methods that determined that the 82 sequences belonged to DENV-1 genotype V (online Technical Appendix Figure 1). We detected 145 mutations in 143 polymorphic sites, and we found 42 sites that were negatively selected with ≥2 of the assayed methods. We found no positively selected sites. The selection analysis resulted in an overall dN/dS of 0.05, which is consistent with our previous work on the 2009 outbreak sequences, where the overall dN/dS ratio was also <1 (*4*).

We found 18 amino acid substitutions in the E protein. Using the Meta-CATS (metadata-driven comparative analysis tool for sequences) statistical analysis tool (*5*), we found that 4 of these substitutions (S338L, R394K, V428L, and V436I) divided our sequences into 2 groups (p<0.01): 1 was related to the 2009 Buenos Aires outbreak and the other to sequences from Brazil (2010–2013). Additionally, we found within the Brazil group a subgroup of 10 sequences containing unique substitutions D235E, K325R, and K361R (p<0.01). Amino acid substitutions are not located on reported epitope positions. Glycosylation sites Asn-67 and Asn-153 were conserved in all the sequences we analyzed.

We performed phylodynamic and phylogeographic analyses on a total of 198 DENV-1 genotype V E-protein gene sequences from the Americas (82 obtained in this study and the rest retrieved from the NCBI Dengue Virus Resource) to analyze the origin, dynamics, and temporal-spatial diffusion process of the 2016 outbreak. We inferred that the most recent common ancestor was located in the Caribbean, with the highest state probabilities in the British Virgin Islands (0.54) and Puerto Rico (0.35), by the end of 1975 (95% HPD 1972–1979). The mean rate of nucleotide substitution was 6.95×10^{-4} substitutions/site/year (95% HPD 5.87×10^{-4}–8.11×10^{-4}), similar to previous reports (*6*–*8*).

A maximum clade credibility tree revealed the co-circulation of 2 lineages in Buenos Aires during 2016, characterized by the 4 amino acid substitutions described (online Technical Appendix Figure 2). One of the lineages has an inferred origin in Venezuela around 1999 (95% HPD 1998–2004) and arrived in Argentina around 2007 (95% HPD 2006–2007). We found 43 sequences from the 2016 outbreak, along with sequences from the 2009 outbreak

previously described by our laboratory, in this lineage. The inferred origin for the second lineage is the British Virgin Islands around 1984 (95% HPD 1982–1985), later arriving in Brazil around 1999 (95% HPD 1996–2000). This lineage comprises 39 sequences from the 2016 outbreak, arranged in 3 subclades originated during 2012–2015.

Conclusions

Our continuous work in DENV diagnosis, surveillance, and research enabled us to characterize the serologic status of the population of Buenos Aires in 2009. We found that an unusually high percentage of the population had secondary DENV infections in what was considered at the time a non-endemic area; therefore, we proposed that cryptic DENV circulation causing inapparent infections might be affecting this area (9). We also described the phylogenetic and phylogeographic characteristics of the first DENV-1 outbreak in 2009; the circulating virus clustered in a monophyletic group within genotype V, which is the most predominant DENV-1 genotype in the Americas (10). In this study, we found that the virus in the 2016 outbreak is also genotype V DENV-1; surprisingly, phylogenetic studies revealed that 2 lineages were circulating concurrently. Both identified lineages are related to sequences from different neighboring countries, and we observed no monophyletic groups local to Buenos Aires or other provinces of Argentina. The co-circulation of 2 DENV lineages was recently reported in Brazil (11,12).

Our data suggest that DENV-1 is established in Buenos Aires and that this densely populated area is changing from one with sporadic outbreaks to an endemic zone. Of note, other arboviruses transmitted by the same mosquito vector, such as Zika and chikungunya, caused autochthonous cases in northern provinces of Argentina in 2016. We believe that the Buenos Aires metropolitan area is now a susceptible area for the emergence of other DENV serotypes, as well as other viruses transmitted by the same vector. Public health authorities should develop stronger prevention and control strategies to avoid future arbovirus outbreaks.

Ethics Statement

These results are part of a study approved by the Medical Ethics and Research Committees of "Ricardo Gutiérrez" Children's Hospital, Buenos Aires, Argentina (IRB No. 10.46). We did not obtain informed consent because patient information was anonymized and deidentified before analysis.

Acknowledgments

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Technical Appendix

Sequencing, analysis, and annotation

We extracted viral RNA from sera by standard EZ1 BioRobot protocol (EZ1 Virus Mini Kit v2.0; QIAGEN, Valencia, CA, USA) and stored it at −20°C. We amplified the coding sequence of the E protein using OneStep RT-PCR Kit (QIAGEN) according to the manufacturer's instructions. The primer pairs were 790: 5′-GGAGACTTGGGCTTTGCGACACCC-3′ / 1491: 5′-GCCCAGTTCTAGGTGAGCAG-3′; 1208: 5′-GTGGACAGAGGCTGGGGTAATGGC-3′ / 2257: 5′-GTCCAAGAAACACCGCTGAACA-3′ and 2125: 5′-AAGCAACCGCCCGAGGAG-3′ / 2904: 5′-GTAGGAGTCACGCAATTTCAACCA-3′, as previously described (*1*,*2*).

We analyzed sequences in an ABI3500 genetic analyzer and obtained consensus sequences by compiling overlapping reads with SeqScape Software v2.7 (Applied Biosystems, Foster City, CA, USA), reference sequence AF226687.2). We inferred amino acid sequences using the universal code by BioEdit software (*3*). The sequences were submitted to GenBank (KX768338-KX768419).

To determine how natural selection acted on the viruses analyzed in our laboratory, we measured the ratio of non-synonymous (dN) to synonymous (dS) substitutions per site (dN/dS) by Datamonkey and DNAsp v.5 (DNA Sequence Polymorphism) (*4*,*5*). The Datamonkey analysis was performed using the following codon-based maximum likelihood (ML) methods: Single Likelihood Ancestor Counting (SLAC), Fixed Effects Likelihood (FEL) and Random Effects Likelihood (REL) at the specified significance levels (p-value <0.1 and Bayes factor 50) (*6*). We used Meta-CATS statistical analysis tool available in NIAID Virus Pathogen Database and Analysis Resource (ViPR) online to identify amino acid positions that significantly differ between the sequences obtained in this study (*7*,*8*).

Genotyping

To genotype the sequences obtained from the 2016 outbreak, we included a total of 260 DENV sequences in the analysis. Of them, 82 sequences were obtained in this study, 27 DENV-1 sequences were previously reported by our laboratory and 134 sequences of different DENV-1 genotypes were retrieved from the NCBI Dengue Virus Resource (*9*). Sequences were aligned with MUSCLE (*10*).

We used jModelTest v.0.1.1 to determine that the GTR+G+I model was the appropriate nucleotide substitution model for the sequence alignment (*11*). We obtained phylogenetic inferences using Neighbor Joining (Mega software v.5.2.2 (*12*)), ML (PhyML v.20120412 software (*13*)) and Bayesian criteria (MrBayes software v.3.2.3 (*14*)). Branch support of the Neighbor Joining tree was evaluated by non-parametric bootstrapping with 1,000 pseudo-replica. We evaluated the convergence of Monte Carlo Markov Chains (MCMC) implemented in the Bayesian criteria with split frequencies ≤0.01 and in TRACER v.1.6 with an effective sample size (ESS) >200; the initial 10% of the run length was discarded as burn-in. We visualized consensus trees with FigTree v.1.4.3.

Phylodynamic and phylogeographic analyses

To evaluate the origin of the 2016 outbreak, we performed a discrete phylogeographic analysis on 198 E-protein sequences of American DENV-1 genotype V, including the 82 sequences obtained in this study. We associated each sequence to the country of probable infection and the year of collection. We used Bayesian coalescent-based methods implemented in the BEAST package v.1.8.2 (*15*). All BEAST run logs were analyzed with TRACER after evaluating the convergence of the MCMC as described in the previous section. The evolutionary model TIM3+I+G, the molecular clock Lognormal relaxed clock (uncorrelated), and the demographic model GMRF Bayesian Skyride used in BEAST package were selected by Bayes Factor. The maximum clade credibility tree was visualized by TreeAnnotator.

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Technical Appendix Table. Representative samples of DENV-1 collected from dengue patients during 2016 outbreak in Buenos Aires, Argentina

| Sample	Collection date	Patient age, y/sex	Location of infection	Amino acid and position								
				180	222	235	325	338	361	394	428	436
				A	S	D	K	S	K	R	V	V
HNRG102741	2016 Jan 19	36/M	Argentina, Misiones	–	–	–	–	–	–	–	–	–
HNRG106053	2016 Feb 29	65/F	Argentina, Buenos Aires	V	–	–	–	–	–	–	–	–
HNRG102917	2016 Jan21	7/F	Paraguay	V	–	–	–	–	–	–	–	–
HNRG106056	2016 Feb 29	10/M	Argentina, Buenos Aires	–	–	–	–	–	–	–	–	–
HNRG103674	2016 Feb 1	45/M	Argentina, Misiones	–	–	–	–	–	–	–	–	–
HNRG104323	2016 Feb 10	14/F	Argentina, Buenos Aires	–	–	–	–	–	–	–	–	–
HNRG104496	2016 Feb 11	63/F	Argentina, Buenos Aires	–	–	–	–	–	–	–	–	–
HNRG106613	2016 Mar 3	28/F	Brazil	–	–	–	–	–	–	–	–	–
HNRG106477	2016 Mar 2	39/M	Brazil	–	–	–	–	–	–	–	–	–
HNRG105181	2016 Feb 19	30/F	Argentina, Buenos Aires	–	–	–	–	–	–	–	–	–
HNRG104715	2016 Feb 15	31/F	Argentina, Buenos Aires	–	–	–	–	–	–	–	–	–
HNRG105546	2016 Feb 23	12/M	Argentina, Jujuy	–	–	–	–	–	–	–	–	–
HNRG105346	2016 Feb 22	18/F	Argentina, Buenos Aires	–	–	–	–	–	–	–	–	–
HNRG104228	2016 Feb 10	33/F	Argentina, Buenos Aires	–	–	–	–	–	–	–	–	–
HNRG105220	2016 Feb 19	31/F	Argentina, Buenos Aires	–	–	–	–	–	–	–	–	–
HNRG106428	2016 Mar 2	50/M	Argentina, Entre Ríos	–	–	–	–	–	–	–	–	–
HNRG104546	2016 Feb 12	28/F	Argentina, Buenos Aires	–	–	–	–	–	–	–	–	–
HNRG108046	2016 Mar 11	47/F	Argentina, San Luis	–	–	–	–	–	–	–	–	–
HNRG105079	2016 Feb 18	65/F	Argentina, Buenos Aires	–	–	–	–	–	–	–	–	–
HNRG105107	2016 Feb 18	41/M	Argentina, Buenos Aires	–	–	–	–	–	–	–	–	–
HNRG105184	2016 Feb 19	24/F	Argentina, Buenos Aires	–	–	–	–	–	–	–	–	–
HNRG104768	2016 Feb 15	45/F	Argentina, Buenos Aires	–	–	–	–	–	–	–	–	–
HNRG104789	2016 Feb 15	17/F	Argentina, Entre Ríos	–	–	–	–	–	–	–	–	–
HNRG107088	2016 Mar 7	32/F	Bolivia	–	–	–	–	–	–	–	–	–
HNRG107216	2016 Mar 7	30/M	Bolivia	–	–	–	–	–	–	–	–	–
HNRG105173	2016 Feb 19	43/F	Argentina, Buenos Aires	–	–	–	–	–	–	–	–	–
HNRG104763	2016 Feb 15	24/M	Argentina, Buenos Aires	–	–	–	–	–	–	–	–	–
HNRG104983	2016 Feb 17	18/F	Argentina, Buenos Aires	–	–	–	–	–	–	–	–	–
HNRG104743	2016 Feb 15	41/F	Argentina, Tucumán	–	–	–	–	–	–	–	–	–
HNRG105512	2016 Feb 23	33/F	Brazil	–	–	–	–	–	–	–	–	–
HNRG105620	2016 Feb 19	11/F	Brazil	–	–	–	–	–	–	–	–	–
HNRG105686	2016 Feb 24	35/F	Brazil	–	–	–	–	–	–	–	–	–
HNRG107304	2016 Mar 8	27/F	Argentina, Santiago del Estero	–	–	–	–	–	–	–	–	–
HNRG104502	2016 Feb 11	11/M	Argentina, Buenos Aires	–	–	–	–	–	–	–	–	–
HNRG102566	2016 Jan 14	1/M	Argentina, Misiones	–	–	–	–	–	–	–	–	–
HNRG106110	2016 Feb 29	25/F	Argentina, Buenos Aires	–	–	–	–	–	–	–	–	–
HNRG105949	2016 Feb 26	34/F	Brazil	–	T	–	–	–	–	–	–	–
HNRG105906	2016 Feb 26	43/F	Brazil	–	T	–	–	–	–	–	–	–

	Collection date	Patient age, y/sex	Location of infection	Amino acid and position								
				180	222	235	325	338	361	394	428	436
Sample				A	S	D	K	S	K	R	V	V
HNRG105062	2016 Feb 18	40/F	Argentina, Buenos Aires	–	T	–	–	–	–	–	–	–
HNRG106045	2016 Feb 29	78/M	Argentina, Buenos Aires	–	T	–	–	–	–	–	–	–
HNRG104513	2016 Feb 11	52/M	Brazil	–	T	–	–	–	–	–	–	–
HNRG104075	2016 Feb 5	30/M	Brazil	–	T	–	–	–	–	–	–	–
HNRG104047	2016 Feb 4	35/M	Brazil	–	–	–	–	–	–	K	–	–
HNRG103430	2016 Jan 28	41/M	Argentina, Buenos Aires	–	–	E	R	L	R	K	L	I
HNRG103011	2016 Jan 22	29/M	Paraguay	–	–	E	R	L	R	K	L	I
HNRG104255	2016 Feb 10	30/M	Argentina, Buenos Aires	–	–	E	R	L	R	K	L	I
HNRG104580	2016 Feb 12	36/F	Argentina, Buenos Aires	–	–	E	R	L	R	K	L	I
HNRG103330	2016 Jan 27	51/F	Argentina, Misiones	–	–	E	R	L	R	K	L	I
HNRG103696	2016 Feb 1	26/F	Argentina, Buenos Aires	–	–	E	R	L	R	K	L	I
HNRG104100	2016 Feb 5	45/M	Argentina, Buenos Aires	–	–	E	R	L	R	K	L	I
HNRG108252	2016 Mar 11	42/M	Argentina, Misiones	–	–	E	R	L	R	K	L	I
HNRG105486	2016 Feb 23	39/F	Argentina, Buenos Aires	–	–	E	R	L	R	K	L	I
HNRG104247	2016 Feb 10	13/M	Argentina, Misiones	–	–	E	R	L	R	K	L	I
HNRG103745	2016 Feb 1	20/M	Brazil	–	–	–	–	L	–	K	L	I
HNRG107955	2016 Mar 10	13/F	Brazil	–	–	–	–	L	–	K	L	I
HNRG104048	2016 Feb 4	42/F	Argentina, Buenos Aires	–	–	–	–	L	–	K	L	I
HNRG108235	2016 Mar 11	52/M	Argentina, Formosa	–	–	–	–	L	–	K	L	I
HNRG104492	2016 Feb 11	29/F	Argentina, Buenos Aires	–	–	–	–	L	–	K	L	I
HNRG104842	2016 Feb 16	31/F	Argentina, Buenos Aires	–	–	–	–	L	–	K	L	I
HNRG104865	2016 Feb 16	43/M	Argentina, Buenos Aires	–	–	–	–	L	–	K	L	I
HNRG102652	2016 Jan 18	NA/M	Argentina, Formosa	–	–	–	–	L	–	K	L	I
HNRG106691	2016 Mar 3	52/M	Argentina, Córdoba	–	–	–	–	L	–	K	L	I
HNRG105776	2016 Feb 25	51/F	Argentina, Santiago del Estero	–	–	–	–	L	–	K	L	I
HNRG107038	2016 Mar 7	63/M	Brazil	–	–	–	–	L	–	K	L	I
HNRG106060	2016 Feb 29	23/F	Argentina, Buenos Aires	–	–	–	–	L	–	K	L	I
HNRG103677	2016 Feb 1	58/M	Argentina, Buenos Aires	–	–	–	–	L	–	K	L	I
HNRG105835	2016 Feb 25	19/F	Argentina, Santiago del Estero	–	–	–	–	L	–	K	L	I
HNRG105076	2016 Feb 18	13/M	Argentina, Buenos Aires	–	–	–	–	L	–	K	L	I
HNRG104922	2016 Feb 16	42/F	Argentina, Buenos Aires	–	–	–	–	L	–	K	L	I
HNRG104694	2016 Feb 15	12/M	Argentina, Buenos Aires	–	–	–	–	L	–	K	L	I
HNRG110386	2016 Apr 13	30/F	Argentina, Buenos Aires	–	–	–	–	L	–	K	L	I
HNRG106373	2016 Mar 1	49/F	Argentina, Chaco	–	–	–	–	L	–	K	L	I
HNRG105836	2016 Feb 25	39/M	Argentina, San Luis	–	–	–	–	L	–	K	L	I
HNRG105691	2016 Feb 24	28/M	Argentina, Entre Ríos	–	–	–	–	L	–	K	L	I
HNRG105481	2016 Feb 23	46/M	Argentina, Misiones	–	–	–	–	L	–	K	L	I
HNRG103646	2016 Feb 1	28/F	Argentina, Buenos Aires	–	–	–	–	L	–	K	L	I
HNRG102532	2016 Jan 14	16/M	Paraguay	–	–	–	–	L	–	K	L	I
HNRG103191	2016 Jan 25	43/F	Paraguay	–	–	–	–	L	–	K	L	I
HNRG105214	2016 Feb 19	63/M	Argentina, Buenos Aires	–	–	–	–	L	–	K	L	I
HNRG105212	2016 Feb 19	30/F	Argentina, Buenos Aires	–	–	–	–	L	–	K	L	I
HNRG107068	2016 Mar 7	42/F	Argentina, Córdoba	–	–	–	–	L	–	K	L	I
HNRG102886	2016 Jan 21	17/M	Argentina, Misiones	–	–	–	–	L	–	K	L	I

*Singleton variations are not shown. Letters indicate substitution, dashes no substitution. A, alanine; D, aspartic acid; E, glutamic acid; I, isoleucine; K, lysine; L, leucine; R, arginine; S, serine; T, threonine; V, valine; NA, not available.
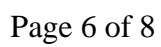
Technical Appendix Figure 1. Phylogenetic Bayesian consensus tree. Posterior probabilities > 0.70 are shown on the nodes (4E+6 generations sampling every 4E+3 generations). Sequences included in the analysis are named with GenBank accession number. Sequences reported in this study are highlighted in blue.
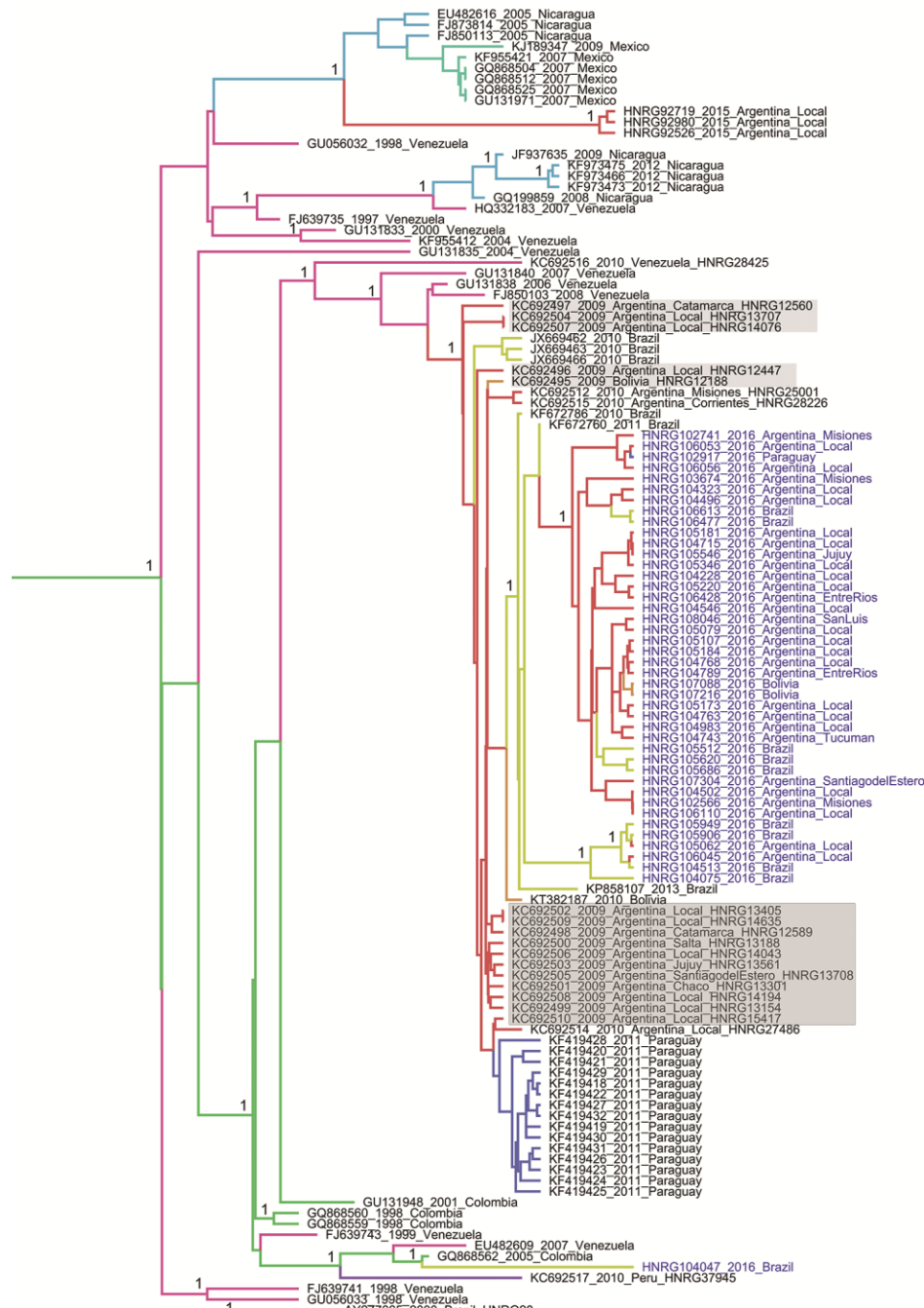
Technical Appendix Figure 2. Maximum clade credibility tree obtained by discrete phylogeographic analysis of the coding sequence from the envelope protein of dengue virus type 1 genotype V isolates

from the Americas. Posterior probabilities equal to 1 are shown on the nodes (2 x 108 generations sampling every 2 x 104 generations). Sequences downloaded from GenBank are named as accession number_year of collection_source country, and sequences obtained in the virology laboratory at Hospital de Niños R. Gutiérrez are named as HNRGnumber_year of collection_source country(_Argentinean province). Blue indicates sequences reported in this study; gray indicates sequences from 2009.